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I, DAVID DANIEL CLARKE, ASSISTANT DIRECTOR PATENT SERVICES, hereby certify that the annexed is a true copy of the Provisional specification as filed on 25 February 1994 in connection with Application No. PM 4114 for a patent by CENTRAL SYDNEY AREA HEALTH SERVICE filed on 25 February 1994.

I further certify that the annexed specification is not, as yet, open to public inspection.

WITNESS my hand this Eighth day of March 1995.

DAVID DANIEL CLARKE
DELEGATE OF COMMISSIONER OF PATENTS

AUSTRALIA

PATENTS ACT 1990

CENTRAL SYDNEY AREA HEALTH SERVICE

PROVISIONAL SPECIFICATION

Invention Title:

Method and Device for the Provocation of Upper or Lower Airway Narrowing and/or the Induction of Sputum

The invention is described in the following statement:

METHOD AND DEVICE FOR THE PROMOTION OF AIR PASSAGE NARROWING AND/OR THE INDUCTION OF SPUTUM

FIELD OF THE INVENTION

The present invention relates to a method and device useful to provoke airway narrowing and/or the induction of sputum. More particularly the invention relates to the use of dry powdered substances to induce a change in the osmolarity of the airways to induce narrowing and/or induction of sputum.

BACKGROUND ART

Asthma is a chronic inflammatory disease of the airways resulting in, amongst other things, a desquamated epithelium, an abnormal number of inflammatory cells and an increased thickness of the basement membrane. The airways of asthmatics are sensitive to a wide variety of chemical, physical, and allergenic stimuli. This sensitivity is manifested by narrowing of the airways and a reduction in the forced expiratory volume in one second (FEV_1).

Bronchial provocation testing, measuring changes in FEV_1 in response to inhaled stimuli, is well established as a technique for identifying and assessing the severity of airway hyperresponsiveness in persons suspected of having asthma.

The most commonly used provocative agents are histamine and methacholine that act directly on specific receptors in the airways causing bronchial smooth muscle contraction. Challenges with these agents have a high negative predictive value but a low specificity for asthma when performed in a random population. Recently there have been problems with availability of these agents and accreditation for their use in humans. Currently the only product approved for human use by the Federal Drug Administration in the USA is Provoline (Hoffman La Roche) which is methacholine chloride. Its cost is approximately $50 per ampoule.

The pathological changes associated with asthma have the potential to affect ion transport and the movement of water to and from the airway lumen. It was recognised in the 1970's that the airways of asthmatics are sensitive to the loss of water from the respiratory tract when large volumes of air need to be humidified to body conditions during exercise and hyperventilation. As a result of this the present inventor put forward the hypothesis that an increase in the concentration of sodium and chloride ions in the airways was the mechanism whereby exercise provoked an attack of asthma. Although this hypothesis has never been proven directly, it prompted the development of a bronchial provocation test using hyperosmolar aerosols of sodium chloride and dextrose. In the last 10 years the inventor's laboratory has developed and standardised a bronchial provocation test using hyperosmolar aerosols of saline generated by an ultrasonic nebuliser. This test is now used in Pulmonary Function Laboratories throughout Australia and is listed in the Medical Benefits Schedule Book. This challenge test is also included in the report of the working party of the European Community for Steel and Coal. It has recently been recommended by the Bronchial Provocation Committee of the International Study of Asthma and Allergy in Children.

The airway response to hyperosmolar saline appears to be highly specific for asthma and the tests have a high positive predictive value with acceptable sensitivity. Hyperosmolar challenge also identifies subjects who would suffer exercise-induced asthma. Challenge with 4.5% saline is cheap, easy to carry out has good patient acceptability and the responses are reproducible.

Hyperosmolar challenge appears to be a very useful technique to evaluate the drugs used in the treatment of asthma. The airway responses to hyperosmolarity are markedly inhibited by steroids, beta-agonists, sodium cromoglycate, and nedocromil. Many asthmatics taking

bronchitis. In recent years the technique has been used with patients with HIV who are suspected of having *Pneumocystis carinii* which causes pneumonia and needs to be treated. Sputum analysis in patients suspected of having tuberculosis is also known as a simple technique to look for the disease.

By increasing the osmolarity of the airway surface liquid water moves towards the lumen of the airway. This movement of water and the mucociliary clearance induced by hyperosmolarity stimulates sputum production. The problem associated with this treatment is similar to the problem associated with the hyperosmolar challenge for the determination of airway narrowing. An expensive nebuliser is required to carry out the procedure.

BRIEF DISCLOSURE OF THE INVENTION

The present invention consists in a method for attempting to provoke airway narrowing in a subject comprising the steps of (a) causing the subject to inhale into the airways an effective amount of a substance capable of altering the osmolarity of airway surface liquid in the subject, which substance is in the form of a dispersible dry powder containing an effective proportion of particles of a respirable size, and (b) measuring in the subject a parameter indicative of the resistance to air flow of the subject's airways.

In another aspect the present invention consists in a method for inducing sputum. When a subject inhales into their airways an effective amount of a substance capable of altering the osmolarity of airway surface liquid, the substance being in the form of a dispersible dry powder containing an effective proportion of particles of a respirable size.

In a still further aspect the present invention consists in a rupturable container containing an effective quantity of a substance capable of altering the osmolarity of airway surface liquid in a subject, the substance being

aerosol steroids long-term may be expected to be negative to the challenge. There is also evidence from pathology that sensitivity to hyperosmolar challenge can be used as an indirect measure of airway inflammation. For example mast cell and eosinophil number in biopsies from the airways of asthmatics correlates with sensitivity to 4.5% NaCl.

The precise mechanism whereby hyperosmolarity leads to airway narrowing is not known. At present it is thought that mast cell mediators and neuropeptides from sensory nerves are released in response to this stimulus. The evidence to support this contention comes primarily from the demonstration, and confirmation by all investigators, that the airway responses to hyperosmolarity are markedly inhibited by specific antihistamines. The only evidence in support of neuropeptide comes from work in animals. It shows indirectly that C-fibres are stimulated by hyperosmolarity and there is an increase in microvascular permeability that can be accounted for by the release of neuropeptides.

It is believed that hyperosmolar challenge is an attractive alternative to the bronchial provocation tests currently used in epidemiology. The advantage in using this challenge in epidemiology is in its high specificity and seemingly high sensitivity for asthma.

The major disadvantage of the hyperosmolar challenge as it is presently practiced using wet aerosols of salt is that it requires the use of a nebuliser which is an expensive piece of apparatus not generally available in the home, schools or the surgeries of general practitioners.

It is also known that wet aerosols of salt can be used for the purpose of inducing sputum in a subject. This technique has been used since the 1970's by physiotherapists to enhance the clearance of secretions from the airways of subjects having cystic fibrosis and

in the form of a dispersible dry powder containing an effective proportion of particles of a respirable size.

The administration of a hyperosomolar challenge to a subject in the form of a dry powder rather than a wet aerosol enables the challenge to be administered through a conventional inhaler rather than through a nebuliser. This is highly advantageous as the inhaler is a very cheap item of equipment and is widely available. It would appear that substantially smaller doses of challenge substance need to be administered in the dry state to achieve a desired response than was required with that substance in the form of a wet aerosol.

As used in this specification, the term "airways" includes both the upper airways of the nose and the lower airways of the lungs. While the invention is particularly applicable in the latter case it is also applicable in the former case for the detection of actual or incipient rhinitis, which may be due to dry air or allergens, and similar conditions. While the invention is hereinafter described with particular reference to the lower airways, this teaching could be applied with equal effect to the airways of the nose.

The substance to be inhaled may be any substance that is biologically compatible with the subject and is capable of altering, normally increasing, the osmolarity of the airway surface liquid of the subject. Preferably the substance is a mineral salt or a sugar, more preferably it is selected from the group comprising sodium chloride, potassium chloride, lactose, mannitol and dextrose. Of the more preferred groups of substances sodium chloride is most preferred for its cheapness, its availability in the required particle size and its biological compatibility.

The substance is required to be inhaled into the airways, usually bronchi, and an effective quantity is required to deposit on the surface of the airways.

Preferably the substance will make contact with the airways surface in the first twelve generations of the airways. For this to happen it is necessary for the inhaled substance to be present initially in a sufficient quantity, for it to be sufficiently dispersible that it can be entrained by the subject's inhaled breath or by a propellant gas, and a sufficient proportion must be of a respirable particle size. The term "respirable particle size" is taken to mean a size that is sufficiently small that the particle will not settle out or impact against the subject's throat rather than be drawn into the airways of the subject's lungs. In practice it has been found that particles of less than about seven microns are respirable.

In the case in which it is desired to induce airway narrowing, such as for testing for asthma, it may be desirable that as much of the powder is of a respirable size as is possible to reduce coughing. By contrast in the case where it is desired to induce sputum it may be desirable to include both respirable particles and non-respirable particles as the latter may induce coughing which will itself assist in the production of sputum. The desired dose in either case will depend upon individual circumstances and will be selected by the supervising medical practitioner as appropriate.

The method for attempting to provoke narrowing may be used for testing subjects for their susceptibility to asthma. In this case the subject may be administered a series of challenges each of a higher dose of the selected substance. After each challenge the subject will be tested for airway narrowing, usually by measuring the forced expiratory volume in 1 second (FEV_1). Other known methods for measuring parameters indicative of airway narrowing could equally well be used. It will be appreciated that in many cases there will be no narrowing which is indicative of a negative propensity for asthma or

rhinitis. In the case of subjects susceptible to asthma there will be a narrowing of the airways proportional to the sensitivity of the subject to the effective administered dose of the substance, that is the dose actually reaching the airway surface. In each case the parameter indicative of resistance to airflow after challenge is compared with the same parameter measured before the challenge to indicate the presence or absence of airway narrowing.

The substance is preferably packaged in a rupturable hard capsule, e.g. gelatin. The capsules preferably contain doses of from 1 to 100 mg, preferably 5 to 40mg in the case of hyperosmotic challenge. In the case of sputum induction higher doses may prove desirable in subjects where airway narrowing is not a concern. In the case where it is desired to induce sputum from asthmatics it may be necessary to premedicate the subject with a beta adrenoreceptor agonist, Intal or Nedocromil sodium before treatment to prevent the airways narrowing.

The present method for the induction of sputum may be used not only to collect sputum for analysis for the presence of viral or microbial pathogens but also in asthmatics to harvest inflammatory cells from the lung. This allows the state of activation of these inflammatory cells to be determined without invasive harvesting of the cells from the subject.

BEST METHOD OF CARRYING OUT THE INVENTION

AIM

To establish the efficacy of using a capsule system to deliver sodium chloride particles for inducing airway narrowing in patients being treated for asthma.

SUBJECTS

Asthmatics aged between 18 - 60 years were recruited from the local community.

All subjects were non smokers, had a baseline forced expiratory volume in one second (FEV_1) greater than 65 %

and a provocation dose of 4.5% saline to cause a 15% fall in lung function (PD_{15}) < 20 mls. Subjects were excluded from the study if they had a chest infection in the previous six weeks. Subjects could not take bronchodilators for six hours before the lab visit, no corticosteroids were taken on the day of the study and no anti-histamines for three-five days before the study day. This study was approved by the Royal Prince Alfred Hospital Ethics Committee and all subjects were required to sign a consent form prior to commencing the study.

METHOD

Experimental design:

On the first visit to the laboratory each subject performed a standard bronchial provocation test with 4.5% saline delivered by an ultrasonic nebuliser. They were included in the study if they had a 20% reduction in FEV_1 provoked by this challenge. They returned to the laboratory on 2 or 3 occasions. A minimum period of 48 hours separated each visit. On each of these occasions they inhaled sodium chloride from the capsule. Eight subjects performed the challenge with Batch 1, six with Batch 1 and Batch 2, and 4 subjects performed the challenge with Batch 2 only.

Preparation and device for delivering powder:

A powder of sodium chloride was prepared by spray drying an aqueous solution and milling it so that particle size was in the respirable range (>7 microns). The powder was produced by Genentech Inc, South San Francisco, California and sent in vials of 400 mg to our laboratory. There were two batches of vials, the first batch was of 4 g and the second batch 20 g. Known amounts (5, 10, 20 and 40 mg) of the dried powdered sodium was packaged in hard gelatin capsules (Gallipot, St Paul, Minnesota 55120) by our laboratory staff. In order to reduce any possibility of re-hydration, this was carried out under controlled air conditions (temperature 20 ± 1 ^{o}C; relative humidity

40%) a Fisons' Intal® Halermatic® device was used for delivering the salt.

Delivery of the powder to the subjects:

The Halermatic was loaded with a capsule containing either 5, 10, 20, or 40 mg of sodium chloride. The capsule was broken and the subject inhaled either once or twice to empty the capsule. The flow rate was measured indirectly by measuring the change in pressure at the mouth (Viggo-Spectromed DTX Disposable Pressure Transducer, Oxnard, CA, USA) during a maximal forced inspiration and values between 40 - 70 litres/min were recorded (Miniwriter Type WTR771A, Watanbe Instruments Corp). The low flow rates are due to the resistance of the device.

Measurement of the response:

- The FEV_1 was measured (Minato Autospirometer AS300, Minato Medical Science Co Ltd, Osaka, Japan) in duplicate, 60 seconds after the administration of the capsule.
- The highest value was taken to calculate the airway response.
- The reduction in FEV_1 for each dose was expressed as a percentage of the value for FEV_1 measured immediately before the challenge.
- For Batch 1 the dose was started at 5 mg and was doubled with each exposure to a culmulative dose of 155 mg (5, 10, 20, 2 x 20, 2 x 20, 2 x 20 mg). When this failed the culmulative dose was increased to 300 mg (20, 2 x 20, 4 x 20, 4 x 20, 4 x 20 mg).
- For Batch 2 the initial dose was usually 5 or 10 mg.
- The subjects performed spirometry for at least 30 minutes following the completion of each challenge to assess spontaneous recovery.
- The response has been expressed in 2 ways. The first is the maximum % fall in FEV_1, the second is the delivered dose of sodium chloride, expressed in

mg, required to provoke a 15% or 20% fall in FEV_1 (PD_{15} or PD_{20}). These values were obtained by linear interpolation from a graph relating % fall in FEV_1 to the dose of sodium chloride delivered.

Measurement of the particle size:

The particle size was measured on a multistage liquid impinger (Astra Pharmaceuticals). This device measures particles in the range of 13 - 6.8 μm, 6.8 - 3.1 μm and <3.1 μm. This device was used to measure the dose of sodium chloride that was in the respirable range (<6.8 μm). To do this 25 ml of sodium chloride of known osmolarity was placed in each of the 3 stages of the impinger. Three 40 mg capsules of sodium chloride from Batch 2 were placed in the Halermatic and the powder was drawn through the impinger via a 'throat' at 60 L/min. The osmolarity of the fluid in the 3 stages was measured. The results of this showed that approximately 40 mg was within the respirable range and this represented 30% of the total dose drawn through the impinger.

RESULTS

The results are presented in the 3 accompanying tables and illustrated (Fig 1) for one subject who received both Batch 1 and Batch 2. Thirteen subjects were accepted into the study on the basis of their challenge with 4.5% saline aerosol. The first 3 subjects received the low and high dose of Batch 1. There were small differences with the response being close to positive in subjects Nos 1 & 3 at the higher dose. Subject No.1 returned for a challenge with Batch 2 and had a positive response with a 20% fall after 146 mg. Subjects Nos 5, 8 & 9 had the high dose of Batch 1 and also had Batch 2. In each subject the response became positive with Batch 2 and the recorded PD_{20} 's were less than 120 mg. In subject Nos. 1, 7 and 12, we performed the test twice with Batch 2 and the results had acceptable reproducibility. The results for subject No.9 are

illustrated in Fig 1, highlighting the difference between the two batches of salt.

Oximetry was performed in order to detect if there was any reduction in arterial oxygen saturation during the challenge. Only one subject had a reduction in saturation of clinical significance and all values remained within the normal range throughout the challenge.

DISCUSSION

The results of this study clearly demonstrate that sodium chloride, delivered from a capsule via a Halermatic device, can provoke airway narrowing in the same asthmatic subjects who are sensitive to the wet aerosol preparation of saline. Although the number of subjects studied is small, there was a good range in the severity of asthma as judged by the PD_{20} to the wet aerosol. There were no untoward side effects, however several subjects found difficulty in inhaling the powder, particularly in the 40 mg dose. We estimated that only 30% of the dose deposited in the lower respiratory tract while the remainder impacted on the device and the throat. Ideally a greater percentage of the dose would have a particle size in the respirable range.

TABLE 1

SODIUM CHLORIDE CAPSULE STUDY DATA										
					WET AEROSOL CHALLENGE					
					4.5% SALINE CHALLENGE					
Subject Number	Name	Height	Age	DOB		Amt neb ml	mg	Max % fall	PD15 ml	PD20 ml
1	AW	189	29	24 Mar 64		28.0	1260.0	58	11.5	14.5
2	JD	176	56	27 Feb 37		20.0	900.0	36	6.1	10.5
3	MM	157	20	25 Jun 73		4.9	220.5	22	1.7	3.3
4	AG	166	23	26 Feb 70		10.0	450.0	30	5.3	6.6
5	NS	167	21	31 May 72		9.0	405.0	33	4.6	5.6
6	DE	191	21	8 Apr 72		21.6	972.0	53	9.8	11.3
7	SB	186	28	6 Nov 65		10	450.0	36	3.5	5.2
8	FM	167	25	29 Oct 68		5.3	238.5	32	2.8	3.4
9	JS	160	26	7 Aug 67		4	180.0	21	2.5	3.7
10	LT	166	25	1 Mar 68		1.9	85.5	21	1.0	1.8
11	LK	173	19	21 Feb 74		19.3	868.5	39	9.6	11.1
12	FMc	163	25	15 Nov 68		23.7	1066.5	24	16.3	20.3
13	CM	180	23	28 Feb 71		23.1	1039.5	27	5.17	16.1

TABLE 2

DRY POWDER CHALLENGE					
	NaCl CAPSULE STUDY Low Dose Batch 1			NaCl CAPSULE STUDY 300mg Batch 1	
	Dose (mg)	Max % fall		Dose (mg)	Max % fall
AW	155	7.0	AW	300	14.7
JD	155	4.3	JD	300	6
MM	195	11.0	MM	300	14
			AG	300	5.6
			NS	300	12.4
			DE	300	6
			SB	300	4.5
			FM	300	5.1

TABLE 3

DRY POWDER CHALLENGE									
	NaCl CAPSULE STUDY Batch 2					NaCl CAPSULE STUDY Batch 2			
	Dose (mg)	Max % fall	PD15 mg	PD20 mg		Dose (mg)	Max % fall	PD15 mg	PD20 mg
AW	150	21	111.0	146	AW	150	23	94.3	126
JD					JD				
MM					MM				
AG	230	27	122.0	155	AG				
NS	150	29	54.5	120.7	NS				
DE	470	22.3	336.0	423	DE				
SB	150	21.4	88.6	125.5	SB	110	20.5	65.9	104.8
FM	40	50	4.0	20.45	FM				
JS	110	21	82.0	106	JS				
LT	30	53	21.2	28.55	LT				
LK					LK				
FMc	610	19	137.2	493.5	FMc	630	22	367	502
CM	670	11			CM				

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

DATED this 25 day of February 1994

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